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to

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STUDIES ON SALIVATION

In our earlier report, we examined the effect of some procedural factors on salivation which was essential to the standardization of the newly developed salivation technique for mice.

The present report incorporates:

1. Expansion of experiments aimed at improving standardization of the salivation procedure.
2. Examination of the possibility to predict the level of base-salivation* by a simple one-day salivation test.
3. Determination of distribution of base-salivations in a herd of mice.
4. Pilot experiments on effects of atropine on salivation of preselected mice.
5. Pilot experiments on effects of metacholine on salivation of preselected mice.
6. Projection of experiments for the near future.

The experiments described in this report were all done in male Ha/ICR Millerton Swiss mice, approximately fifteen to twenty-four weeks of age, and housed six to a cage, each wearing a felt collar with consecutive numbers permanently around its neck. None of the mice used in the respective experiments had prior experience with the salivation technique.

1. On standardization of the salivation procedure: factors affecting salivation in mice - The results of a preliminary experiment, described in Report No. 12, indicated that sex, days and number of measurements during each day are important factors which affect the rate of base-salivation in mice. These results were based on data from male and female mice, salivated once, twice or three times a day over four consecutive days.

We will now report on the results of four additional experiments which were performed to examine the effects of the same factors but with an extended number of levels, on base-salivation. These experiments were designed to examine the reproducibility of our earlier conclusions. The results will

*Base-salivation is a term used to indicate the amount of saliva obtained over a ten-minute period with bit No. 5, expressed in mm boundary displacement on a cotton strip.

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also aid towards a more standardized base-salivation procedure; the experimental design might serve as a basic procedure by which future drug and smoke treatments can be examined effectively; and finally, these experiments would ultimately lead to the availability of a herd of mice with known salivation levels.

This study consisted of four identical experiments with different mice. For each experiment, the mice were assigned at random to three groups of ten mice each. The animals of Group I were studied three times daily; those of Group II, four times daily, and those of Group III, five times daily for four consecutive days. Group I mice were studied once in the forenoon, noon and afternoon; those of Group II, twice in the forenoon and afternoon, and those of Group III, once at noon, and twice in the forenoon and afternoon, in an attempt to reduce variability of salivation data that might be due to the time during the day or interval between measurements within a day. The time lapse between consecutive salivation measurements was approximately ninety minutes.

Immobilization of the mice for salivation was achieved by fitting their collars to stancions. Salivation was initiated using a type No. 5 bit. The amount of saliva produced during a ten-minute period was measured and the data recorded.

The data were analyzed by means of the analysis of variance technique in which the entire study is regarded as one replication of a split-pilot experiment in which Factor A (Experiments) has four levels (Experiments 1, 2, 3 and 4) and Factor B (Times) has twenty levels (Day 1, Measurement 1; Day 1, Measurement 2---etc.).

Tables 1, 2, 3 and 4 present the base-salivation results of the four experiments, and Tables 5, 6 and 7, the analyses of variance pertaining to the 5 x daily, 4 x daily and 3 x daily data, respectively.

With five measurements a day, a highly significant difference in base-salivation was found among experiments, whereas this was not found to be the case among experiments with the three or four measurements daily. Closer examination of the data of five daily measurements revealed that this differential result among experiments could well be explained by the variance of the initial salivation level of the mice in one of the experiments.

Further statistical analysis indicated that, regardless of the salivation frequency, approximately 50% of the error variance in a typical measurement could be attributed to differences between individual mice, thus confirming the earlier finding that considerable differences in base-salivation levels exist between individual mice which are reproducible over consecutive sali-

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vation measurements.

Base-salivation increases to a highly significant degree with time. This increase in base-salivation over time can, to a large extent, be attributed to a significant linear increment in the daily salivation levels during four consecutive days which is valid regardless of the number of salivation measurements during the day. In contrast to the above, this increase can, only to a small extent, be explained by rank order of the salivation measurements during the day and is valid with the three and four times daily measurements, but not with the five times daily measurements. These results indicate that under the experimental conditions of four consecutive days, we have not as yet reached our goal, which is to establish conditions for obtaining a base-salivation plateau over time.

In conclusion, these experiments establish firmly that approximately 50% of the error variance can be attributed to differences between individual mice, which strongly suggests that preselection of mice will contribute to efficiency of subsequent experiments.

2. On the prediction of salivation level by a one-day test - The experimental data from the experiments reported above were used to examine whether the "true" salivation level of individual mice could be predicted by their salivation performance on the first experimental day. The "true" salivation level was estimated by the mean salivation rate over four days.

As a first approach to answer this question, correlation and regression coefficients were determined between the mean salivation rate on the first day and the mean salivation level of four days, on groups of mice salivated three, four and five times daily. The results are presented in Table 8.

Since the correlation coefficients were found to be very high (.78 to .88), the data were examined in detail. Within each salivation frequency (3, 4 or 5 times daily), the mice were classified under three sub-groups, representing "low", "medium" and "high" salivations, as based on their mean salivation over four days. The "low" salivation range was from 0 to 16.7; the "medium", from 16.7 to 25, and the "high", above 25.0 mm of boundary displacement.

Tables 9 and 10 present the results obtained which show that the salivation level of "low"- and "high"-salivating mice can be predicted by a one-day salivation test with good accuracy, whereas only reasonable accuracy can be predicted in the "medium" salivation group.

3. Base-salivation in a population of mice - The results reported under Sections 1 and 2 show that base-salivation of individual mice over time is repro-

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ducible and that salivation performance can be predicted to a considerable degree by the one-day salivation test. The above justifies the preselection of mice on the basis of their salivation performance by the one-day salivation test in the hope of obtaining more uniform starting material for subsequent salivation studies.

Five hundred male Ha/ICR Millerton Swiss mice, 12 to 14 weeks old and weighing 18 to 25 gm, were used. After two weeks of adaptation to their cages and collars, the mice were examined by the one-day salivation test. The test consisted of determining on each mouse, four base-salivations and calculating the mean of the 2nd, 3rd and 4th salivation (salivation index). The time lapse between consecutive salivations was ninety minutes, and the mice were returned to their respective cages during that period.

Three hundred and eighty-five of the five hundred mice finished the one-day salivation test. One hundred and fifteen mice were lost, primarily due to the breaking of their necks during the first salivation. The salivation indices for this herd of mice ranged from 1.3 to 51.3 mm, with a mean salivation index of 17.65 mm and a median of 15.3 mm.

The distribution of salivation indices for the above herd is graphically presented in Figure 1. It clearly shows that the salivation indices are not distributed normally and that a relatively large number of mice have a low salivation index, as compared to only a small number possessing a high salivation index.

Based on our findings that the "true" salivation level is predictable, this herd of "typed" mice will be used in experiments to be reported subsequently, with the purpose of reducing the amount of random variation in the data.

4. Atropine and salivation - The subcutaneous administration of atropine sulfate to mice (0.05 mg/kg in 0.05 ml of isotonic saline solution) ten minutes prior to the start of salivation was found previously to inhibit significantly the base-salivation in mice (Report No. 12, pg. 5).

The pilot experiment to be reported here was planned to expand the above finding. Fifty male Ha/ICR Millerton Swiss mice (sixteen to eighteen weeks old) were systematically obtained from the pre-typed herd. Twenty-five mice were randomly drawn from the population with a "salivation index" of 10 to 25 mm ("Intermediate High" [IH]), and the other twenty-five were randomly drawn from the population with a salivation index of 25 mm and above ("High High" [HH]). Their individual body weights were recorded, and the mice in these two salivation populations were subsequently assigned to one of five treatment groups, housed individually, but in association with each other, for one day prior to the actual experiment. Treatment Groups II, III,

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IV and V received a subcutaneous injection with atropine sulfate of a dose of 0.66, 2, 6.6 and 20 mg in 0.05 ml of isotonic saline ten minutes prior to salivation. Thus, each treatment group consisted of ten mice with five mice from IH and five mice from HH, respectively.

The mice were salivated four times during the experimental day, twice in the forenoon, and twice in the afternoon, with a two-hour time lapse between consecutive salivations. The first salivation served as an indicator for the salivation level of individual mice during that particular day. Ten minutes prior to the actual start of the second salivation, the mice were injected subcutaneously with atropine sulfate, returned to their cages and salivated subsequently. The third and fourth base-salivations were performed two and four hours after the second salivation.

The results of the experiment are presented in Table 11. An examination of the validity of preselection of mice for salivation was performed by statistical analysis of any systematic differences in salivation in the first measurement between IH and HH salivators. The analysis of variance of this effect is presented in Table 12. The highly significant difference in salivation performance in the first measurement between IH and HH salivators established that preselection three weeks prior to this experiment had been effective.

The data show further that atropine sulfate administered subcutaneously ten minutes prior to salivation exerts a highly significant inhibition on base-salivation. The dose range (.66 mg to 20 mg of atropine sulfate per animal) was apparently too narrow to show any clear dose-dependence of this inhibition.

Note further that the salivation inhibition by atropine sulfate is only short term. Any significant inhibition due to atropine sulfate could not be observed two or four hours after injection. Further experiments are required to more firmly establish the above findings over a wider dose range.

5. Metacholine and salivation - The subcutaneous injection of metacholine in mice had previously been found to significantly stimulate base-salivation. The experiment to be reported here was planned to broaden in a quantitative sense our knowledge on this aspect.

Forty male Ha/ICR Millerton Swiss mice, fifteen to seventeen weeks old, were drawn at random from among the low salivators in the mice herd described under Section 3. Their salivation indices ranged between 1.3 and 7.6 mm. The mice were assigned at random to one of four groups of ten mice each. Group I served as controls, whereas Groups II, III and IV received 0.083, 0.833 and 8.33 mg of metacholine subcutaneously in 0.1 ml

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of double-distilled water, respectively.

The four groups of mice were salivated four times within one day, twice in the forenoon, and twice in the afternoon, the time lapse between salivations being approximately ninety minutes. The first, second and fourth salivations served as controls, whereas injections were made at the moment of start of the third salivation. The results of this experiment are presented in Table 13. The analyses of variance are presented in Table 14.

These tables show that 8.33 mg of metacholine (s.c.) is lethal to mice under our experimental conditions. Base-salivation was stimulated to a highly significant degree and in a dose-related fashion by subcutaneous injection of metacholine. The salivation measurements performed ninety minutes after injection showed, on the other hand, a significantly lowered salivation performance in the metacholine-treated groups, suggesting an important after-effect to drug treatment. Additional experiments are required to examine the various findings in more quantitative detail.

6. Projection of experiments for the near future - For the near future, we hope to:

a. improve on standardization of base salivations;

b. examine in depth the effect of metacholine on salivation;

c. more firmly establish the effect of subcutaneously-injected atropine on salivation;

d. initiate pilot experiments on the effect of smoke on salivation.

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Table 1
(Experiment 1)

The Effect of Number of Salivation Measurements in Four Consecutive Days on Base-Salivation of Ha/ICR Millerton Swiss Male Mice (Approximately Eighteen Weeks Old)

Group	I				II					III							Means
	1	2	3	sub-total	1	2	3	4	sub-total	1	2	3	4	5	sub-total	Total	
Day 1	110	178*	268	556	248	159	216	174	797	347	302	208	351	422	1640	2983	24.9
Day 2	270	208	203	681	237	203	346	268	1054	419	371	465	378	251	1884	3619	30.2
Day 3	254	261	136	651	187	161	260	305	913	507	459	406	516	465	2353	3917	32.6
Day 4	216	255	318	789	211	297	281	364	1153	617	572	651	614	589	3043	4985	41.5
Total	850	902	925	2677	883	820	1103	1111	3917	1890	1704	1730	1859	1727	8910	15504	
Means	21.3	22.6	23.1	22.3	22.1	20.5	27.6	27.8	24.5	47.3	42.6	43.3	46.5	43.2	44.6	32.3	

* Number of mm salivated in ten minutes by a group of ten mice.

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Table 2
(Experiment 2)

The Effect of Number of Salivation Measurements in Four Consecutive Days on Base-Salivation of Ha/ICR Millerton Swiss Male Mice (Approximately Twenty Weeks Old)

Group	I				II					III							Means
	1	2	3	sub-total	1	2	3	4	sub-total	1	2	3	4	5	sub-total	Total	
Day 1	194*	386	330	910	148	206	233	175	762	167	177	144	147	189	824	2496	20.8
Day 2	426	359	330	1115	240	239	259	253	991	291	201	162	271	238	1163	3269	27.2
Day 3	363	403	276	1042	213	318	299	346	1176	211	212	137	159	192	913	3131	26.1
Day 4	413	339	409	1161	203	210	224	213	850	284	198	277	252	300	1311	3322	27.7
Total	1396	1487	1345	4228	804	973	1015	987	3779	953	788	722	829	919	4211	12214	
Means	34.9	37.2	33.6	35.2	20.1	24.3	25.4	24.7	23.7	23.8	19.7	18.1	20.7	23.0	21.1	25.5	

* Number of mm salivated in ten minutes by a group of ten mice.

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Table 3
(Experiment 3)

The Effect of Number of Salivation Measurements in Four Consecutive Days on Base-Salivation of Hs/ICR Millerton Swiss Male Mice (Approximately Twenty-one Weeks Old)

Group	I				II					III							Means
	1	2	3	sub-total	1	2	3	4	sub-total	1	2	3	4	5	sub-total	Total	
Day 1	151*	277	300	728	95	244	298	246	883	116	189	183	152	211	851	2462	20.5
Day 2	226	328	388	942	194	327	283	293	1097	295	207	261	199	344	1306	3345	27.9
Day 3	374	428	410	1212	250	368	332	238	1188	297	282	285	243	357	1464	3864	32.2
Day 4	263	281	368	912	260	271	273	277	1081	321	208	271	243	241	1284	3277	27.3
Total	1014	1314	1466	3794	799	1210	1186	1054	4249	1029	886	1000	837	1153	4905	12948	
Means	25.4	32.9	36.7	31.6	20.0	30.3	29.7	26.4	26.6	25.7	22.1	25.0	20.9	28.8	24.5	27.0	

* Number of mm. salivated in ten minutes by a group of ten mice.

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Table 4
(Experiment 4)

The Effect of Number of Salivation Measurements in Four Consecutive Days on Base-Salivation of Ha/ICR Millerton Swiss Male Mice (Approximately Twenty-three Weeks Old)

Group	I				II					III							Means
	1	2	3	sub-total	1	2	3	4	sub-total	1	2	3	4	5	sub-total	Total	
Day 1	145*	195	208	548	123	161	173	221	678	177	255	207	206	226	1071	2297	19.1
Day 2	331	241	192	764	217	297	270	200	984	277	249	153	282	320	1281	6029	50.2
Day 3	212	232	242	686	259	289	279	273	1100	304	238	289	279	323	1433	3219	26.8
Day 4	177	210	279	666	226	303	235	275	1039	379	351	302	334	394	1760	3465	28.9
Total	565	878	921	2664	825	1050	957	969	3801	1137	1093	951	1101	1263	5545	15010	
Means	21.6	22.0	23.0	22.2	20.6	26.3	23.9	24.2	23.8	28.4	27.3	23.8	27.5	31.6	27.7	31.3	

* Number of mm salivated in ten minutes by a group of ten mice.

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Table 5

Analysis of Variance of Base-Salivation of Ha/ICR Millerton Swiss Male Mice (Nineteen to Twenty-four Weeks Old) Over a Period of Four Consecutive Days When Measured Five Times Each Day

Source	D F	S S	M S	F cal	F 95%	F 99%
Total	799	374,345				
Units	39					
Experiments (E)	3	65,143	21,714	6.13**	2.96	4.60
Replications (Rep)	9	21,060	2,340	0.66	2.31	3.26
Error (a)	27	95,712	3,545			
Sub-units						
Times (T)	19	27,616	1,453	6.96**	1.52	1.79
Days (D)	3	23,354	7,785	37.25**	2.61	3.78
Linear	1	22,825	22,825	109.21**	3.84	6.64
Linear deviation	2	529	264	1.26	3.00	4.61
Measurements (M)	4	2,296	574	2.75*	2.37	3.32
D x M	12	1,966	164	0.78	1.75	2.19
E x T	57	21,830	383	1.83**	1.52	1.79
E x D	9	11,678	1,298	6.21**	1.94	2.51
E x M	12	2,068	172	0.82	1.75	2.19
E x D x M	36	8,084	225	1.07	1.52	1.79
Error (b)	684	142,984	209			

* Significant at the 5% level.

** Significant at the 1% level.

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Table 6

Analysis of Variance of Base-Salivation of Ha/ICR Millerton Swiss Male Mice (Nineteen to Twenty-four Weeks Old) Over a Period of Four Consecutive Days When Measured Four Times Each Day

Source	D F	S S	M S	F cal	F 95%	F 99%
Total	639	229,498				
Units	39					
Experiments (E)	3	883	294	0.09	2.96	4.60
Replications (Rep)	9	19,679	2,187	0.66	2.31	3.26
Error (a)	27	89,255	3,306			
Sub-units						
Times (T)	15	9,887	659	3.56**	1.75	2.19
Days (D)	3	5,822	1,941	10.49**	2.61	3.78
Linear	1	3,321	3,321	17.95**	3.84	6.64
Linear deviation	2	2,481	1,240	6.70**	3.00	4.61
Measurements (M)	3	3,402	1,134	6.13**	2.61	3.78
D x M	9	663	74	0.40	1.94	2.51
E x T	45	9,897	220	1.19	1.52	1.79
E x D	9	2,338	260	1.41	1.94	2.51
E x M	9	2,277	253	1.37	1.94	2.51
E x D x M	27	5,282	196	1.06	1.52	1.79
Error (b)	540	99,897	185			

* Significant at the 5% level.

** Significant at the 1% level.

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Table 7

Analysis of Variance of Base-Salivation of Ha/ICR Millerton Swiss Male Mice (Nineteen to Twenty-four Weeks Old) Over a Period of Four Consecutive Days When Measured Three Times Each Day

Source	D F	S S	M S	F cal	F 95%	F 99%
Total	479	213,899				
Units	39					
Experiments (E)	3	15,760	5,253	2.08	2.96	4.60
Replications (Rep)	9	18,065	2,007	0.80	2.31	3.26
Error (a)	27	68,071	2,521			
Sub-units						
Times (T)	11	10,379	944	4.82**	1.75	2.19
Days (D)	3	4,019	1,340	6.84**	2.61	3.78
Linear	1	2,495	2,495	12.73**	3.84	6.64
Linear deviation	2	1,411	705	3.60*	3.00	4.61
Measurements (M)	2	1,035	518	2.64	3.00	4.61
D x M	6	5,325	887	4.53**	2.10	2.80
E x T	33	24,071	729	3.72**	1.52	1.79
E x D	9	2,881	320	1.63	1.94	2.51
E x M	6	1,985	331	1.69	2.10	2.80
E x D x M	18	19,205	1,067	5.44**	1.52	1.79
Error (b)	396	77,553	196			

* Significant at the 5% level.

** Significant at the 1% level.

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Table 8

Relationship between the Mean Salivation Rate of the First Experimental Day and Mean Salivation Level, Expressed in Terms of Correlation Coefficients and Regression Coefficients

No. Measurements/Day	Correlation Coefficient (r)	Regression Coefficient (b)
3	.83	.97
4	.78	.83
5	.88	.96

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Table 9

Relationship between Predicted and Actual Salivation Level for High, Medium and Low Salivators, Expressed in Actual Numbers

3 x

		Predicted			
		Low	Medium	High	Total
Actual	Low	10	4	0	14
	Medium	3	6	2	11
	High	1	1	9	11
	Total	14	11	11	36

4 x

		Predicted			
		Low	Medium	High	Total
Actual	Low	12	3	0	15
	Medium	3	7	4	14
	High	0	4	6	10
	Total	15	14	10	39

5 x

		Predicted			
		Low	Medium	High	Total
Actual	Low	9	1	0	10
	Medium	1	9	3	13
	High	0	3	14	17
	Total	10	13	17	40

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Table 10

Relationship between Predicted and Actual Salivation Level for High, Medium and Low Salivators, Expressed in Percentages

No. Measurements/Day	Low	Medium	High	Overall
3	.71	.55	.82	.69
4	.80	.50	.60	.64
5	.90	.69	.82	.80

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Table 11

The Effect of Subcutaneous Injections of Atropine Sulfate on the Base-Salivation of Ha/ICR Millerton Swiss Male Mice (Sixteen to Eighteen Weeks Old)

		Measurement No.							
		I		II		III		IV	
			sub-total		sub-total		sub-total		sub-total
Group I (control)	Low	67*		78		133		144	
	High	115		104		186		208	
	sub-total		182		182		319		352
Group II (.66 mg)	Low	77		15		142		261	
	High	142		14		268		237	
	sub-total		219		29		410		498
Group III (2 mg)	Low	57		12		174		177	
	High	66		4		89		222	
	sub-total		123		16		263		399
Group IV (6.6 mg)	Low	39		3		98		101	
	High	80		9		187		103	
	sub-total		119		12		285		204
Group V (20 mg)	Low	74		7		84		78	
	High	111		3		86		255	
	sub-total		185		10		170		333
	Total		828		249		1447		1786

* Figure represents the total amount of salivation in mm by five mice.

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Table 12

The Effect of Preselection of Ha/ICR Millerton Swiss Male Mice (Sixteen to Eighteen Weeks Old) on Subsequent Salivation Performance

Source	D F	S S	M S	F cal	F 95%	F 99%
Total	47	5,252				
Groups	4	748	187	2.01	2.61	3.83
Preselection	1	800	800	8.60**	4.08	7.31
Interaction	4	166	41	0.44	2.61	3.83
Error	38	3,538	93			

* Significant at the 5% level.

** Significant at the 1% level.

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Table 13

The Effect of Subcutaneously Administered Metacholine on Base-Salivation of Ha/ICR Millerton Swiss Male Mice (Fifteen to Seventeen Weeks Old)*

Measurement No.	M 1	M 2	M 3	M 4
Group I (control)	183**	127	164	159
Group II (0.083 mg)	141	188	373	143
Group III (0.833 mg)	157	103	812	97
Group IV (8.33 mg)	154	196	all died	---

* Totals for ten mice in each group.

** Figure represents total amount of base-salivation of ten mice.

Examination of Drug Effect

	Baseline (B) $\frac{(M\ 1 + M\ 2)}{2}$	Response (M 3 - B)	Residual Effect (M 4 - B)
Group I (control)	155	9	4
Group II (0.083 mg)	165	208	-22
Group III (0.83 mg)	130	682	-33

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Table 14

Analysis of Variance

The Effect of Metacholine Administered Subcutaneously at the Start of the Salivation Test on Base-Salivation of Ha/ICR Millerton Swiss Male Mice (Fifteen to Eighteen Weeks Old)

Source	D F	S S	M S	F cal	F 95%	F 99%
Total	29	37,749				
Between doses	2	23,907	11,954	23.3**	3.35	5.49
Within doses	27	13,842	513			

* Significant at the 5% level.

** Significant at the 1% level.

Analysis of Variance

The Effect of Metacholine Administered Subcutaneously on Base-Salivation of Ha/ICR Millerton Swiss Male Mice Salivated Ninety Minutes after Injection

Source	D F	S S	M S	F cal	F 95%	F 99%
Total	29	3,262				
Between doses	2	1,502	751	11.5**	3.25	5.49
Within doses	27	1,760	65			

* Significant at the 5% level.

** Significant at the 1% level.

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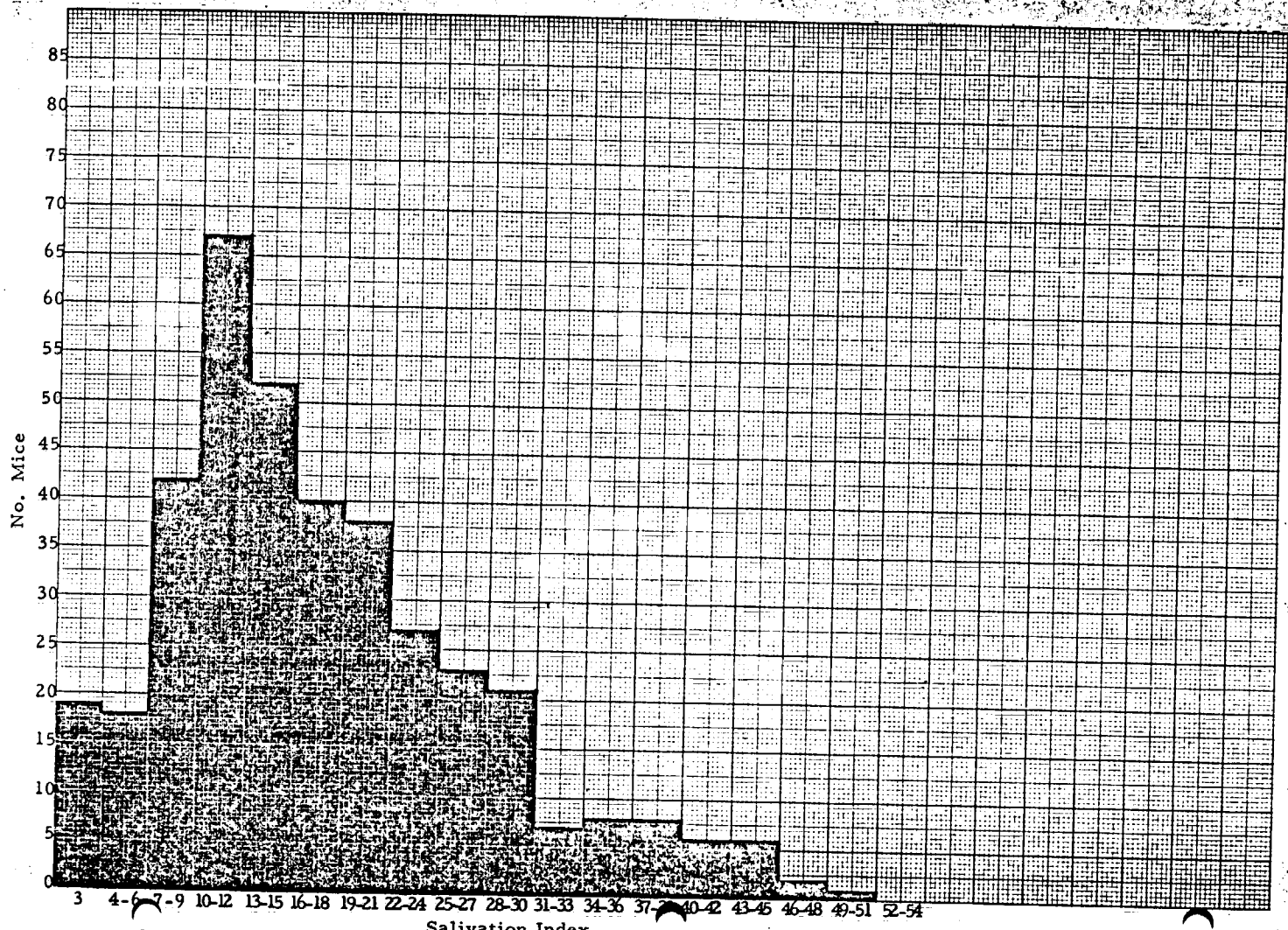
Source: https://www.industrydocuments.ucsf.edu/docs/ggyl0000

Figure 1
Frequency Distribution of Salivation Indices in a Herd of 385 Ha/ICR Millerton Swiss Male Mice (Twelve to Fourteen Weeks Old)



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Source: https://www.industrydocuments.ucsf.edu/docs/ggyl0000